201-14433

ESCO Company Limited Partnership

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April 25, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program

RE: EPA HPV Registration No.:

Dear Administrator Whitman,

ESCO Company Limited Partnership is electronically submitting the enclosed proposed test plan and robust summaries for the HPV Challenge Program, AR-201. The test plan and robust summaries are being submitted for the chemical category designated as the "keto acid" category. This keto acid category includes the following two chemicals:

Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl], (C.A.S. No. 5809-23-4), and

Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl], (C.A.S. No. 54574-82-2).

This electronic submission includes this cover letter in Adobe Acrobat format (file name: Cover Letter for Keto Acid Submission.pdf), the keto acid robust summaries in Adobe Acrobat format (file name: Keto Acid Category Robust Sumaries.pdf), and the proposed keto acid test plan in Adobe Acrobat format (file name: Keto Acid Category Test Plan.pdf).

Please post these submissions on the EPA HPV Challenge web site.

If you have any questions, please call me at 231-727-6459 or my e-mail address is Bkatje@escocompany.com.

Sincerely,

Bruce Katje
Regulatory Compliance Manager

Attachments

EPA HPV Challenge
Keto Acid Category Test Plan

ESCO Company Limited Partnership
2340 Roberts Street
Muskegon, Michigan 49443

The Keto Acid Category

Color Former Name	Chemical Name	C.A.S. Number
EtKeto Acid	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]	5809-23-4
BuKeto Acid	Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl	54574-82-2

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Category Definition

1. Identification of Category Members

The EPA High Production Volume (HPV) Challenge identified the following chemical as a high production volume chemical:

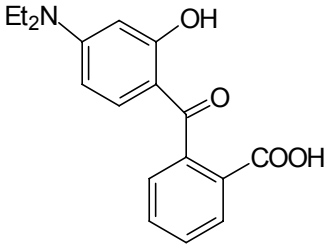
Keto Acid Name	Chemical Name	C.A.S. Number
EtKeto	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]	5809-23-4

The EPA guidance document on the development of chemical categories defines a category as "a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. The similarity may be based on a common functional group."

The following chemical is very similar to EtKeto in molecular structure:

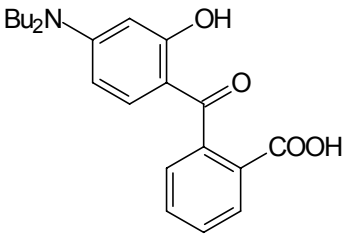
Color Former Name	Chemical Name	C.A.S. Number
BuKeto	Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl	54574-82-2

BuKeto Acid is not identified by the EPA as an HPV chemical, but the structure and the physiochemical and toxicological properties are so similar to EtKeto Acid that it is being included with EtKeto in the keto acid category. The chemical structure diagrams of these two keto acids are shown below.



ETKETO

Formula: $C_{18}H_{19}NO$
MW: 313



BUKETO

Formula: $C_{22}H_{27}NO_4$
MW: 369

2. Category Analysis

The keto acid category members have closely related chemical structures. The keto acids in this category are weak acids with the only differences in the stuctures being the

functional group on the nitrogen. EtKeto Acid has basically the same structure as BuKeto Acid, except the ethyl group attached to the nitrogen is replaced with a butyl group on BuKeto Acid.

3. Test Plan Matrix

The keto acid category test plan matrix that is shown in Table 1 was developed after a review of the existing data available. The matrix is arranged by category members in columns and the screening data endpoints in rows. The table indicates how data are provided for each endpoint.

4. Test Plan Rationale

For each of the screening data endpoints, including physical and chemical data, environmental fate and pathways, ecotoxicity, and toxicologoical data, the rationale for using the category approach is included in the following discussion.

Table 1: Test Plan Matrix for the Keto Acid Category

Screening Endpoints	EtKeto Acid (5809-23-4)	BuKeto Acid (54574-82-2)
Physical and Chemical Data		
Melting Point	A	A
Boiling Point	NA	NA
Density (Specific Gravity)	A	A
Vapor Pressure	C	A
Partition Coefficient (n-Octanol/Water)	C	A
Water Solubility	C	A
pH Value and pKa Value	C	A
Environmental Fate and Pathways		
Photodegradation	A	A
Stability in Water (Hydrolysis)	A	A
Transportation and Distribution between Environmental Compartments	A	A
Biodegradation	A	A
Ecotoxicity		
Acute Toxicity to Fish	C	A
Acute Toxicity to Aquatic Invertebrates	C	A
Toxicity to Aquatic Plants (e.g. Algae)	C	A
Toxicological Data		
Acute Oral Toxicity	C	A
Acute Dermal Toxicity	C	A
Repeated Dose Toxicity	C	A
Gene mutation	C	A
Genetic Toxicity – Chromosomal Aberrations	C	A
Toxicity to Reproduction	C	A
Developmental Toxicity/Teratogenicity	C	A^1

Key: A = Endpoint fulfilled with data
A^1 = Endpoint fulfilled by Toxicity to Reproduction Test data
C = Endpoint fulfilled by other category members data
NA = Test not applicable

4.1 Physical and Chemcial Data

Listed in Table 2 are the values for the physical and chemical data endpoints for the keto acid category. All of the data listed can be found in the robust summaries for the keto acid category. The boiling points were not measured because the keto acids in this category are solids at room temperature and melt at temperatures above 201°C. No boiling point data has been generated for these keto acids. The vapor pressure measured for BuKeto Acid is very low, as it is a solid; EtKeto Acid would have a similar vapor pressure. The water solubility of BuKeto Acid was measured and found to be very limited; as would be expected also for EtKeto Acid based on the similar chemical structure. The solubility increases with increased pH. Because the pKa value could not be calculated due to limited solubility for BuKeto Acid in water, EtKeto Acid would be expected to have similar results because of limited solubility. No further physical and chemical tests are planned for the keto acid category.

Table 2: Physical and Chemical Data

Screening Endpoints	EtKeto Acid (5809-23-4)	BuKeto Acid (54574-82-2)
Physical and Chemical Data		
Melting Point	201 - 204ºC	184ºC
Boiling Point	NA	NA
Density (Specific Gravity)	1.179 g/ml	1.179 g/ml
Vapor Pressure		13 Pa at 20°C
Partition Coefficient (n-Octanol/Water)		Log P_{ow} > 5.003 at pH 5.0 Log P_{ow} = 2.670 at pH 7.0 Log P_{ow} = 1.645 at pH 9.0
Water Solubility		0.000137 kg/M^3 at 30°C and pH 5.0 0.0298 kg/M^3 at 30°C and pH 7.0 1.645 kg/M^3 at 30°C and pH 9.0
pH Value and pKa Value		Not soluble in water enough to perform the test

4.2 Environmental Fate and Pathways

Listed in Table 3 are the values for the environmental fate and pathway endpoints for the keto acid category. All of the data listed can be found in the robust summaries for the keto acid category. The photodegradation and hydrolysis endpoints for the keto acids were estimated with the EPA model, EPIWin. Because the keto acids are solids, the photodegradation pathway is not a very likely scenario for degradation. Very little of the keto acids dissolve in water, so hydrolysis is also not a very likely route of degradation. The most likely route is adsorption to the soil and biodegradation. The biodegradation results from testing Buketo Acid shows that it is not readily biodegradable, which confirms the model results. Because EtKeto Acid is so similar to BuKeto Acid structurally, we would expect that EtKeto is not readily biodegradable either. The EPIWin Model predicts that EtKeto Acid would not be readily biodegradable.

Another test that confirmed the information from the modeling is the adsorption/desorption to soil on BuKeto Acid. BuKeto Acid was strongly absorbed on to the soils tested, and it did not readily desorb from the soils. This result is consistent with the EPWin modeling information. Because EtKeto is structurally similar to BuKeto, it is expected that EtKeto Acid would have similar biodegradation results, and because all of the other endpoints have adequate data, no further environmental fate and pathways tests or estimations are planned for the keto acid category.

Table 3: Environmental Fate and Pathways

Screening Endpoints	EtKeto Acid (5809-23-4)	BuKeto Acid (54574-82-2)
Environmental Fate and Pathways		
Photodegradation	EPIWin Model Half-life ($t^{1/2}$) = 0.05 days	EPIWin Model Half-life ($t^{1/2}$) = 0.05 days
Stability in Water (Hydrolysis)	EPIWin Model Half-life ($t^{1/2}$) = 38 days	EPIWin Model Half-life ($t^{1/2}$) = 15 days
Transportation and Distribution between Environmental Compartments	EPIWin Model Air: 0.0002% Water:19.9% Soil: 78.4% Sediment: 1.74% Persistence: 44 days	EPIWin Model Air: 0.02% Water:30.2% Soil: 69.6% Sediment: 0.2% Persistence: 18 days
Biodegradation		BuKeto Acid did not biodegrade during the 28 day study.

4.4 Ecotoxicity

Listed in Table 4 are the values for the ecotoxicity endpoints for the keto acid category. All of the data listed can be found in the robust summaries for the keto acid category. The data provided for BuKeto Acid shows that it is mildly toxic to fish within its solubility range and mildly inhibitory to alga growth within its solubility range. EtKeto Acid, based on its similar chemical structure is expected to also be mildly toxic to fish and alga growth. Some further tests that were completed on BuKeto Acid include a prolonged toxicity study on rainbow trout, a respiration inhibition test on activated sludge, a toxicity study of BuKeto Acid on earthworms, and a toxicity test of BuKeto Acid on the growth of higher plants. The prolonged toxicity to rainbow trout confirmed the toxicity of BuKeto Acid to fish. The BuKeto Acid earthworm study, respiration inhibition test, and the higher plant growth study showed no adverse effects on the earthworm, the activated sludge or the growth of plants from BuKeto Acid. Because EtKeto is structurally similar to BuKeto, it is expected that EtKeto Acid would have similar results, so no further ecotoxicity testing is planned for the keto acid category.

Table 4: Ecotoxicity

Screening Endpoints	EtKeto Acid (5809-23-4)	BuKeto Acid (54574-82-2)
Ecotoxicity		
Acute Toxicity to Fish		96 h LC_{50} = 16.3 p.p.m.
Acute Toxicity to Aquatic Invertebrates		24 h EC_{50} value = 65.8 p.p.m. 48 h EC_{50} value = 36.3 p.p.m.
Toxicity to Aquatic Plants (e.g. Algae)		EC_{50} = 25.73 p.p.m.

4.5 Toxicological Data

Listed in Table 5 are the values for the toxicological endpoints for the keto acid category. All of the data listed can be found in the robust summaries for the keto acid category. The data provided for acute oral toxicity is consistent between the keto acids. In addition to dermal toxicity, BuKeto Acid was also tested for skin irritation (rabbit), eye irritation (rabbit), and skin sensitization (guinea pig). EtKeto Acid was also tested for skin irritation (rabbit). All of these tests show the keto acids in this category show little or no irritation to the skin or eyes or sensitization to the skin. The repeated dose result for BuKeto Acid is consistent with this pattern of the keto acids in this category, which shows mild toxicity at the medium and greatest dose tested. The *In vivo* gene mutation tests (*In Vivo* Rat Liver Unscheduled DNA Synthesis Assay) on BuKeto Acid was negative even though BuKeto Acid was clastogenic *in vitro* when tested for such effects, to toxic concentrations, in the presence of S9 mix with an established Chinese hamster ovary (CHO) cell line. The gene mutation test for BuKeto Acid was negative. The reproduction test on BuKeto Acid was based on the OECD Method 415, which showed some mild signs of toxicity including a moderate reduction in body weight gain with a concomitant slight reduction in food consumption at 1000 mg/kg/day in males only. There were no notable effects seen in 50 mg/kg/day or 250 mg/kg/day in males or at any dose level in females. The reproduction test was carried on for 10 weeks prior to mating and carried through to 21 days post partum. Based on the results of this test no further developmental tests were conducted. Because of the structural similarity of EtKeto to BuKeto, it is expected that EtKeto would have similar toxicological results. The keto acids in this category show a clear pattern of low toxicological concern, so no further toxicological testing is planned for the keto acid category.

Table 5: Toxicological Data

Screening Endpoints	EtKeto Acid (5809-23-4)	BuKeto Acid (54574-82-2)
Toxicological Data		
Acute Oral Toxicity	LD_{50} > 5.0 g/kg	LD_{50} > 5.0 g/kg
Acute Dermal Toxicity		LD_{50} > 2.0 g/kg
Repeated Dose Toxicity		NOEL > 250 mg/kg/day in males NOEL > 1000 mg/kg/day in females
Gene mutation		Negative
Genetic Toxicity – Chromosomal Aberrations		"In the presence of S9 mix BuKeto Acid was a potent inducer of chromosomal aberrations when tested at toxic concentrations of 20 and 30 µg/ml. This response was dose related. There was no evidence that BuKeto Acid induced chromosomal aberrations in the absence of the S9 mix."
Genetic Toxicity – In Vivo		Negative
Toxicity to Reproduction		NOEL = 50 mg/kg/day
Developmental Toxicity/Teratogenicity		See above result

5. Test Plan Conclusion

EtKeto Acid and BuKeto Acid have closely related chemical structures. The keto acids in this category are weak acids with the only differences in the stuctures being the functional group on the nitrogen. EtKeto Acid is basically the same as BuKeto Acid, except the ethyl group attached to the nitrogen is replaced with a butyl group on BuKeto Acid. All of the data from the robust summaries for the keto acid category show a similar pattern of physical and chemical properties, and a similar pattern of little or mild adverse effects from environmental fate data, ecotoxicity data, and toxicological data.

The data provided in the robust summaries show a pattern that is consistent with the close molecular structure of the keto acids in this category. Both EtKeto Acid and BuKeto Acid are intermediates used in the production of color formers. Both of these intermediates are used as closed system intermediates for our purposes, therefore ther is no exposure outside our faclity for these intermediates.

The data help confirm the validity of the category. No further new testing is planned for the keto acid category.

EPA HPV Challenge
Keto Acid Category Robust Summaries

ESCO Company Limited Partnership
2340 Roberts Street
Muskegon, Michigan 49443

The Keto Acid Category

Color Former Name	Chemical Name	C.A.S. Number
EtKeto Acid	Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]	5809-23-4
BuKeto Acid	Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl	54574-82-2

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Physical and Chemical Elements

1. Melting Point

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	2002
Remarks	None

Results

Melting Point (°C)	201 - 204°C
Decomposition	No
Sublimation	No
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completes melting point testing on each batch of EtKeto Acid produced.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Chemical Testing Guideline No. 102
GLP (Yes/No)	No
Year	1991
Remarks	None

Results

Melting Point (°C)	457.5°K (184.3 °C)
Decomposition	No
Sublimation	No
Remarks	A color change was recorded at 446°K

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Inveresk Research International, "Physico Chemical Testing with DBMAP-Keto Acid," May 30, 1991

Other

None

2. Boiling Point

The two Keto Acids in this Keto Acid category are solids at room temperature and melt at temperatures above 201°C. No boiling point data has been generated for these Keto Acids.

3. Density (Specific Gravity)

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	ESCO Company Quality Assurance Laboratory Method
GLP (Yes/No)	No
Year	2002
Remarks	None

Results

Specific Gravity	1.179 g/mL
Temperature (°C)	20°C
Remarks	None

Conclusions

Remarks: The ESCO Company Quality Assurance Laboratory completed specific gravity determinations on EtKeto Acid.

Data Quality

Remarks: None

References

None

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Chemical Testing Guideline No. 102
GLP (Yes/No)	No
Year	1991
Remarks	None

Results

Specific Gravity	1.179 g/mL
Temperature (°C)	21°C
Remarks	None

Conclusions

Remarks: None.

Data Quality

Remarks: None

References

Inveresk Research International, "Physico Chemical Testing with DBMAP-Keto Acid," May 30, 1991

Other

None

4. Vapor Pressure

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Vapor pressure value listed in Inveresk Research International Toxicokinetic Assessment
GLP (Yes/No)	No
Year	2001
Remarks	None

Results

Vapor Pressure Value	13 Pa at 20°C
Temperature (°C)	At 20°C
Decomposition	No

Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Inveresk Research International, "Toxicokinetic Assessment of BuKeto Acid," January 10, 2001.

Other

None

5. Partition Coefficient

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Chemical Testing Guidelines No. 107
GLP (Yes/No)	No
Year	1991
Remarks	None

Results

Log P_{ow}	Log P_{ow} > 5.003 at pH 5.0 Log P_{ow} = 2.670 at pH 7.0 Log P_{ow} = 1.645 at pH 9.0
Temperature °C	20°C
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Inveresk Research International, “Physico Chemical Testing with DBMAP- Keto Acid,” May 30,1991.

Other

None

6. Water Solubility

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Test Guideline No. 105
GLP (Yes/No)	No
Year	1991
Remarks	None

Results

Value (mg/L) at temperature °C	0.000137 kg/m^3 at 30°C and pH 5.0 0.0298 kg/m^3 at 30°C and pH 7.0 1.645 kg/m^3 at 30°C and pH 9.0
Description of solubility	Not described
pH Value and concentration at temperature °C	0.000137 kg/m^3 at 30°C and pH 5.0 0.0298 kg/m^3 at 30°C and pH 7.0 1.645 kg/m^3 at 30°C and pH 9.0
pKa Value at 25°C	None provided
Remarks	None

Conclusions

Remarks: None

Data Quality

Remarks: None

References

Inveresk Research International, "Physico Chemical Testing with DBMAP- Keto Acid," May 30,1991.

Other

None

7. pKa Value

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline 112
GLP (Yes/No)	Yes
Year	2001
Remarks	None

Results

pKa Value at 25°C	See remarks and conclusion sections
Remarks	"The aqueous test solutions were insoluble, so an additional amount of co-solvent was used in an attempt to increase solubility of the test substance in the methanol test solution. The extra addition of solvent (> 20%) did not increase the solubility of BuKeto Acid in water. An approximation of the pKa value using pure solvent was determined not to be an appropriate comparison to an aqueous solution found in the natural environment."

Conclusions

Remarks: "BuKeto Acid is not soluble in water even with the addition of a solvent, therefore the OECD 112 Dissociation Guideline Test can not be performed.

Dissociation of BuKeto Acid will not be a significant factor in the natural environment, since it will not dissolve in water at any appreciable levels."

Data Quality

Remarks: None

References

Springborn Laboratories, Inc., "BuKeto Acid – Determination of the Dissociation Constant," September 6, 2001.

Other

None

8. Adsorption/Desorption to Soil

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Draft Document; "Estimation of the Adsorption Coefficient (K_{OC}) on Soil and Sewage Sludge Using High Performance Liquid Chromatography (HPLC)"
GLP (Yes/No)	Yes
Year	1999
Remarks	None

Results

Adsorption Coefficient (K_{OC})	"The adsorption coefficient was determined to be log K_{OC} 2.02."
Remarks	None

Conclusions

Remarks: "The adsorption coefficient was determined to be log K_{OC} 2.02."

Data Quality

Remarks: None

References

Inveresk Research, "DBMAP-Ketoacid Estimation of Adsorption Coefficient (K_{OC}) by HPLC Method," October 8,1999.

Other

None

Environmental Fate and Pathway Elements

9. Photodegradation

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	None

Results

Half-life ($t^{1/2}$)	0.05 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Conclusions

Remarks: The oxidation program predicts the half-life of EtKeto Acid to be very short in air. The AOP program predicts the rate at which the test substance will react with air-borne hydroxyl radicals formed through photochemical reactions. This is the most common type of reaction that organic chemicals undergo in relation to photolysis in air. Although the half-life in air is very short, degradation in air is not expected to be a major degradation pathway since the compound is not particularly volatile.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	None

Results

Half-life ($t^{1/2}$)	0.05 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Conclusions

Remarks: The oxidation program predicts the half-life of BuKeto Acid to be very short in air. The AOP program predicts the rate at which the test substance will react with air-borne hydroxyl radicals formed through photochemical reactions. This is the most common type of reaction that organic chemicals undergo in relation to photolysis in air. Although the half-life in air is very short, degradation in air is not expected to be a major degradation pathway since the compound is not particularly volatile.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

10. Stability in Water

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Half-life ($t^{1/2}$)	38 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	None

Conclusions

Remarks: The aqueous hydrolysis rate program could not calculate a hydrolytic rate constant for EtKeto Acid since it is not classified as an ester, carbamate, epoxide, halmethane, or alkyl halide. Hydrolysis is not likely to be a major pathway for degradation of EtKeto Acid since the solubility of EtKeto Acid in water is so low.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Half-life ($t^{1/2}$)	15 days
Degradation % after	Not provided
Breakdown products	Not provided
Remarks	None

Conclusions

Remarks: The aqueous hydrolysis rate program could not calculate a hydrolic rate constant for BuKeto Acid since it is not classified as an ester, carbamate, epoxide, halmethane, or alkyl halide. Hydrolysis is not likely to be a major pathway for degradation of BuKeto Acid since the solubility of BuKeto Acid in water is so low.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

11. Transport Between Environmental Compartments (Fugacity)

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), (Level III Fugacity Model)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Media	Air, Water, Soil, and Sediment
Estimated Distribution and Media Concentration	<u>Environmental Distribution</u> Air: 0.0002% Water: 19.9% Soil: 78.4% Sediment: 1.74% Persistence: 44 days <u>Waste Water Treatment Removal</u> Air: 0.00% Adsorption: 21.22% Biodegradation: 0.25% Total Removal: 21.47% <u>Environmental Half-Life</u> Air: 0.05 days

	Water: 38.0 days Soil: 38.0 days Sediment: 150.0 days <u>Predicted Parameters</u> Hydrolysis: Can not estimate Atmospheric Oxidation: 35 minutes Biodegradation: Weeks Adsorption: K_{oc} = 277
Remarks	None

Conclusions

Remarks: EtKeto Acid is predicted to bind to soil after entering the natural environment. EtKeto Acid is not predicted to readily biodegrade.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Computer Simulated Model – Estimations Program Interface for Windows (EPIWin), (Level III Fugacity Model)
GLP (Yes/No)	Computer Model
Year	2003
Remarks	Used Simplified Molecular Input Line Entry System (SMILES) to design the chemical structure input.

Results

Media	Air, Water, Soil, and Sediment
Estimated Distribution and Media Concentration	<u>Environmental Distribution</u> Air: 0.02% Water: 30.2% Soil: 69.6% Sediment: 0.2% Persistence: 18 days <u>Waste Water Treatment Removal</u> Air: 0.00% Adsorption: 3.57% Biodegradation: 0.11% Total Removal: 3.68% <u>Environmental Half-Life</u> Air: 0.05 days Water: 15.0 days Soil: 15.0 days Sediment: 60.0 days <u>Predicted Parameters</u> Hydrolysis: Can not estimate Atmospheric Oxidation: 34 minutes Biodegradation: Weeks Adsorption: Strong (K_{oc} = 3205)
Remarks	None

Conclusions

Remarks: BuKeto Acid is predicted to bind significantly to soil after entering the natural environment. BuKeto Acid is not predicted to readily biodegrade.

Data Quality

Remarks: None

References

ESCO Company ran the EPIWin Model on April 24, 2003.

Other

None

12. Biodegradation

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline for Testing of Chemicals No. 301B
Test Type	Aerobic
GLP (Yes/No)	Yes
Year	2001
Contact Time	28 days
Innoculum	Activated Sewage Sludge Bacteria
Remarks	None

Results

Degradation % after time	BuKeto Acid did not biodegrade during the 28 day study.
Results	BuKeto Acid may not be classified as readily biodegradable.
Kinetic	Sodium benzoate attained 60% biodegradation within 28 days
Breakdown Products	No
Remarks	There was no evidence of inhibitory effects under the conditions of this test.

Conclusions

Remarks: “Based on the CO_2 analysis, BuKeto Acid cannot be classified as readily biodegradable under OECD guidelines since it did not biodegrade during the 28 day study.”

Data Quality

Remarks: None

References

Springborn Laboratories, Inc., “BuKeto Acid – Determination of the Biodegradability by CO_2 Evolution Modified Sturm Test,” August 8, 2001.

Other

None

Ecotoxicity Elements

13. Acute Toxicity to Fish

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Determination of Acute Toxicity (LC_{50}) to Rainbow Trout (96 H, Static)
Test Type	Acute toxicity to rainbow trout under static conditions.
GLP (Yes/No)	Yes
Year	1990
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Rainbow trout (*Salmo gairdneri*). Source: Cloan Hatcheries, Auchterarder, Scotland
Exposure period	96 hours
Statistical Methods	"The 96 h LC_{50} value, with 95% confidence limits, will be determined by probit analysis. Where possible 24, 48, and 72 h LC_{50} values will also be calculated. The 96 h LC_{50} may also be determined graphically."
Remarks	None

Results

Nominal Concentrations	0, 62.5, 125, 250, 500, 1000 p.p.m.
Measured Concentrations	0, 1.7, 2.8, 7.1, 8.6, 29.4 p.p.m.
Unit	p.p.m.
Element Value	24 hour LC_{50} = 22.3 p.p.m., 48 hour LC_{50} = 16.3 p.p.m., 72 hour LC_{50} = 16.3 p.p.m., 96 hour LC_{50} = 16.3 p.p.m.
Statistical Results	"The 95% confidence limits were not calculable since the data distribution does not fit the probit model used."
Remarks	None

Conclusions

Remarks: "These results are based on mean measured concentrations of BuKeto Acid. The highest mean measured concentration tested causing no mortalities within the test period was 8.6 p.p.m. The lowest mean measured concentration tested causing any mortalities within the test period was 29.4 p.p.m. The lowest mean measured concentration tested causing 100% mortalities within the test period was 29.4 p.p.m."

Data Quality

Remarks: None

References

Inveresk Research International, "DBMAP KETOACID Determination of Acute Toxicity (LC_{50}) to Rainbow Trout (96 h, Static)," November 12, 1990.

Other

None

14. Prolonged Toxicity to Fish

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline, Fish Juvenile Growth Test – 28 days (November 1994)
Test Type	Prolonged toxicity to rainbow trout under semi-static conditions.
GLP (Yes/No)	Yes
Year	1998
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Species/Strain/ Supplier	Rainbow trout (*Oncorhynchus mykiss*). Source: Selcoth Fish Farm, Moffat, Scotland
Exposure period	28 days
Statistical Methods	"In calculation of EC_{20} values (i.e. the concentration resulting in a 20% decrease in growth rate), the tank

	average growth rates were plotted against concentration to examine the concentration response relationship. A simple regression of growth rate on concentration was fitted to the tank average growth rates. For each interval the EC_{20} was calculated. Berkson's modification was applied to the mortality data prior to analysis (due to lack of fractional mortality). A probit transformation was applied to the modified data for each day separately. The probit transformed data was applied to the modified data for each day separately. The probit transformed data was then subjected to a regression procedure against the logarithmically transformed concentrations of test material. From the fitted models, the LC_{50} values were estimated for each day. As Berkson's modification was applied, no confidence limits have been presented."
Remarks	None

Results

Nominal Concentrations	0, 5, 15.8, 50, 158, and 500 mg/L
Measured Concentrations	0, 0.12, 0.40, 1.32, 4.50, and 19.2 mg/L
Unit	mg/L
Element Value	EC_{20} = 6.5 mg/l at 14-28 days, EC_{20} = 10.5 mg/l at 0-28 days based on mean measured concentrations. EC_{50} for mortality was found to be 14.6 mg/L, 10.4 mg/L and 9.3 mg/L for Days 2,3, and 4 respectively LC_{50} = 14.6 mg/l at 2 days, LC_{50} = 10.4 at 3 days, LC_{50} = 9.3 at 4-28 days.
Remarks	"Analysis of test material concentrations indicated that BuKeto Acid was unstable over the 48 hour test solution replacement period at all test concentrations. This instability may be due to hydrolysis, degradation, or binding to glass or fish."

Conclusions

Remarks: "All fish at 19.2 mg/l died within 4 days of exposure and as such could not be included in the growth rate calculations. The only other mortality during the test period was in one solvent control replicate, where one fish escaped from the tank." The 28 day LC_{50} = 9.3 mg/L

Data Quality

Remarks: None

References

Inveresk Research, "BuKeto Acid - Rainbow Trout, Juvenile Growth Test (28 day, Semi-Static)," December 30, 1998.

Other

None

15. Acute Toxicity to Aquatic Plants (e.g. Algae)

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline for Testing Chemicals No. 201
Test Type	Algae growth inhibition test on BuKeto Acid
GLP (Yes/No)	Yes
Year	1997
Species/Strain # and Source	*Selenastrum capricornutum*, Strain No.: CCAP 278/4, Source: Culture Collection of Algae and Protozoa (CCAP), Ambleside, Cumbria
Element Basis	Cell count/mL, area under the curve, and specific growth rate
Exposure period	72 hours
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Statistical Methods	"The area under the growth curve and growth rate values were analyzed for homogeneity of variance using Levene's test (Levene, 1960) at 1% significance level. If the group variances appeared homogeneous, the area under the curve and growth rate values were analyzed using analysis of variance (ANOVA) techniques. Following ANOVA, pairwise comparisons were performed between control and each measured concentration of test material using Dunnett's procedure (Dunnett, 1964) at 5% significance level. If the group's variances appeared heterogeneous, log or square root

	transformations were used in an attempt to stabilize the variances. If the variances remained heterogeneous Dunn's test (Dunn, 1964), a distribution-free multiple comparisons procedure based on Kruskal-Wallis rank sums, was used. The NOEC was calculated for area under growth curves and average specific growth rates using Dunnett's or Dunn's test at 5% significance level. Tests used in the calculation of the NOEC were 2-tailed. The EC50 value (i.e. the concentration of test material which reduces growth by 50%) was calculated where possible from average specific growth rates and areas under growth curves (estimate of biomass) by probit analysis (Finney, 1971) and is shown in table 3. The fit of the probit model to the area under growth curve and growth rate data was checked via the Pearson chi-square test statistic."
Remarks	None

Results

Nominal Concentrations	0, 1.2, 3.7, 11.1, 33.3, 100 p.p.m.
Measured Concentrations	0, 0.374, 1.311, 12.943, 23.805, 62.155 p.p.m.
Unit	p.p.m.
Element Value	Average Specific Growth Rate ($\mu_{ave/day}$) 0-48 hours EC_{50} = 31.47 p.p.m. NOEC = 1.311 p.p.m. 0-72 hours EC_{50} = 25.73 p.p.m. NOEC = 1.311 p.p.m. Area Under Growth Curve (AUC) 0-48 hours EC_{50} = 8.62 p.p.m. NOEC = 1.311 p.p.m. 0-72 hours EC_{50} = 5.02 p.p.m. NOEC = 1.311 p.p.m.
Statistical Results	95% Confidence Intervals EC_{50} Value Average Specific Growth Rate ($\mu_{ave/day}$) Lower Limit / Upper Limit 0-48 hours 25.14 p.p.m. / 40.85 p.p.m. 0-72 hours 20.88 p.p.m. / 32.32 p.p.m. Area Under Growth Curve (AUC) Lower Limit / Upper Limit 0-48 hours 6.86 p.p.m. / 10.72 p.p.m. 0-72 hours 4.05 p.p.m. / 6.15 p.p.m.
Remarks	"At the higher nominal concentrations, the measured concentrations of BuKeto Acid were acutely affected by the time dependant change in pH observed with algal

	cultures." "It is concluded that the increase in test concentrations observed at the higher levels was a result of increasing solubility as the pH increased."

Conclusions

Remarks: The EC_{50} value at 72 hours is 25.73 p.p.m. The NOEC is 1.311 p.p.m.

Data Quality

Remarks: None

References

Inveresk Research, "BuKeo Acid – Alga, Growth Inhibition Test (72 h, EC_{50})," April 15, 1997.

Other

None

16. Acute Toxicity to Aquatic Invertebrates

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Determination of Acute Toxicity (EC_{50}) to Daphnia (48 H, Static)
Test Type	The acute toxicity (EC_{50}) of BuKeto Acid to Daphnia was determined over a 48 hour period, under static conditions.
GLP (Yes/No)	Yes
Year	1991
Species/Strain/ Supplier	*Daphnia magna.* "They were bred within the laboratory by acyclical parthenogenesis and the individuals used were between 6 and 24 hours old."
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Exposure Period	48 hours
Statistical Methods	"The median effective concentration (EC50) at 24 h and 48 h was determine using probit analysis."

Remarks	None

Results

Nominal Concentrations	0, 62.5, 125, 250, 500, and 1000 p.p.m.
Measured Concentrations (p.p.m.)	0, 4.3, 10.1, 24.3, 48.0, and 97.1 p.p.m.
Unit	p.p.m.
Element Value	24 hour EC_{50} value = 65.8 p.p.m. 48 hour EC_{50} value = 36.3 p.p.m.
Statistical Results	95% Confidence Intervals EC_{50} Value Lower Limit / Upper Limit 24 hours: 55.8 p.p.m. – 77.9 p.p.m. 48 hours: Not calculable since the data distribution does not fit the probit model used. The 48 hour EC_{50} value was also calculated graphically as 36.4 p.p.m.
Remarks	None

Conclusions

Remarks: “Results are based on values of the mean measured concentrations of BuKeto Acid. The highest mean measured concentration tested causing no immobilization within the test period was 24.3 p.p.m.
The lowest mean measured concentration causing any immobilization within the test period was 48.0 p.p.m.
The lowest mean measured concentration tested causing 100% immobilization within the test period was 97.1 p.p.m.”

Data Quality

Remarks: None

References

Inveresk Research International, “BuKeto Acid – Determination of Acute Toxicity (EC_{50}) to Daphnia (48 h, Static),” July 10, 1991.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guidelines for Testing Chemicals No. 202
Test Type	A 21-day semi-static reproduction test of BuKeto Acid with *Daphnia magna* was conducted.
GLP (Yes/No)	Yes
Year	1998
Species/Strain/ Supplier	*Daphnia magna.* "They were bred within the laboratory by acyclical parthenogenesis. The individuals used were between 6 and 24 hours old at test initiation."
Analytical Monitoring	High Performance Liquid Chromatography (HPLC)
Exposure Period	21 days
Statistical Methods	The No Observed Effect Concentration (NOEC) for mean number of offspring produced per reproducing *Daphia* was calculated at 14 days and 21 days using data from all replicate tanks. The cumulative number of offspring per reproducing adult data were analyzed separately for homogeneity of variance using Levene's Test (Leven, 1960) at 1% significance level. As the groups appeared homogeneous, these data were analyzed using analysis of variance (ANOVA) techniques (Snedecor and Cochran, 1980). Using the error of variance from the ANOVA, pairwise comparisons between control and treated groups were performed using one-tailed Dunnett's Test (Dunnett, 1964) at 5% significance level. The NOEC is defined as the highest concentration which is not significantly different from the control at the 5% significance level using the on-tailed Dunnett's test. To calculate the EC_{50} value for reproduction, the mean cumulative number of offspring per producing adult was calculated for each concentration. Using Wadley's adjustment (Finney, 1971) for natural mortality, a probit transformation was applied to these data. Where necessary Berkson's modification was also applied. The probit transformed data were then subjected to a regression procedure against logarithmically transformed concentrations of test material with a Newton-Raphson maximum likelihood iterative

	procedure being made to obtain parameter estimates (Finney, 1971). Persons chi-squared goodness-of-fit statistic was used to assess the fit of the model. From the fitted model, the EC_{50} value was estimated together with the associated confidence limits where possible. The NOEC for immobilization of adult *Daphnia* was calculated by comparing the cumulative number of immobile adult daphnia at each concentration with that observed in the controls using Fisher's Exact Test (Fisher, 1950). To calculate the EC_{50} value for immobilization of adult *Daphnia*, the cumulative number of immobile animals were subjected to probit transformation. Where there were less than two concentrations exhibiting fractional mortality data, Berkson's modification (Berkson, 1953) was applied. The probit transformed data were then subjected to a regression procedure as described above. From the fitted model, the EC_{50} value was estimated together with the associated confidence limits where possible."
Remarks	None

Results

Nominal Concentrations	0, 3.16, 10, 31.6, 100, 316 mg/L
Measured Concentrations (mg/L)	0, 0.36, 1.18, 3.46, 11.79, 35.71 mg/L
Unit	mg/L
Element Value	<u>EC_{50} Values for Immobilization of Adult *Daphnia*</u> 0-14 days EC_{50} > 35.71 mg/L 21 days EC_{50} = 19.16 mg/L <u>EC_{50} Values for Reproduction</u> 14 days EC_{50} = 18.96 mg/L 21 days EC_{50} = 14.47 mg/L "The NOEC for reproduction was calculated as 31.6 mg/L nominal (3.46 mg/L measured) at Day 14 and Day 21." "The NOEC for adult immobilization was calculated as 316 mg/L nominal (35.71 mg/L measured) at Day 0 to Day 2 and 100 mg/L nominal (11.79 mg/L measured) at Day 3 to Day 21."
Statistical Results	95% Confidence Intervals EC_{50} Value for Reproduction

		Lower Limit	Upper Limit
	14 days	12.96 mg/L	49.48 mg/L
	21 days	Not calculable as Berkson's modification was applied to the data.	
Remarks	None		

Conclusions

Remarks: The 21 day EC_{50} Value for Immobilization of Adult *Daphnia* is 19.16 mg/L. The 21 day EC_{50} Value for Reproduction is 14.47 mg/L. The NOEC for immobilization was calculated as 35.71 mg/L at Day 0 to Day 2 and 11.79 mg/L at Day 3 to Day 21.The NOEC for reproduction was calculated as 3.46 mg/L at Day 14 and Day 21.

Data Quality

Remarks: None

References

Inveresk Research, "BuKeto Acid – Daphnia Reproduction Test (21 Day, Semi-Static)," July 2, 1998.

Other

None

17. Toxicity to Terrestrial Organisms

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline for Testing Chemicals No. 207
Test Type	Acute toxicity test to earthworm
GLP (Yes/No)	Yes
Year	1998
Species and Source	*Eisenia foetida andrei*, Source: Blades Biological, Kent
Element Basis	Weight loss, and mortality
Exposure period	14 days

Statistical Methods	"The weights of the individual worms in the test and control vessels were recorded, and the mean and standard deviation determined, for each vessel. The mean body weight for each treatment group was calculated at Day 0 and day 14 and the percentage weight loss calculated. Weights were checked for significance of difference between test and control worms over the 14 day test period, using a one-way analysis of variance."
Remarks	None

Results

Concentrations	0, 1, 10, 100, and 1000 mg/kg
Unit	mg/kg
Element Value	"As there were not mortalities in the test vessels, it is concluded that the LC_{50} of BuKeto Acid to earthworms is greater that 1000 mg/kg under the conditions of the test."
Statistical Results	"The results at the end of the 14 day period showed that BuKeto Acid had no effect on earthworm survival."
Remarks	"As the mortalities in the control vessels by the end of the test were less than 10%, the test is considered valid. No unusual behavior or pathological signs were noted in test and control vessels throughout the test period."

Conclusions

Remarks: "As there were not mortalities in the test vessels, it is concluded that the LC_{50} of BuKeto Acid to earthworms is greater that 1000 mg/kg under the conditions of the test."
"After 14 days the mean changes in weight for the test and control worms were –0.017 g and +0.020 g respectively. This difference in weight change between the test and control worms was found to be statistically significant (P-value from the analysis of variance = 0.0003). Although this would infer that BuKeto Acid has a sub-acute effect at 1000 mg/kg under the test conditions, it should be noted that the worms were not fed during the study and the weight differences may not be biologically significant."

Data Quality

Remarks: None

References

Inveresk Research, "BuKeto Acid – Determination of Acute Toxicity to Earthworms (14 Day, Limit Test)," August 12, 1998.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	The effects of BuKeto Acid upon the germination (emergence) and growth of seedlings of wheat *(Triticum aestivum*), radish (*Raphanus sativus*), and mung bean(*Phaseolus aureus*).
Test Type	Terrestrial Plants, Growth Test
GLP (Yes/No)	Yes
Year	1998
Species, Variety, and Source	Crop / Variety / Source Wheat / Riband / Dods of Haddington Radish / Scarlet Globe / Strawberry Corner Mung Bean / *Phaseolus aureus* / Real foods
Element Basis	"Pots were observed daily for emergence and phytotoxic effects."
Exposure period	"The test was terminated 19 days after sowing for wheat and mung beans and 18 days after sowing for radish. These represent 14 days after at least 50% emergence had been observed in the control pots."
Statistical Methods	" The effect of the test material on emergence (LC_{50}) and growth rate was determined, with 95% confidence limits where possible, by probit analysis. The goodness of fit of the probit model to the data was checked via the Pearson chi-squared test statistic. A significant chi-squared test statistic indicated heterogeneity of discrepancies between the observed and expected values. In these cases, no confidence intervals have been presented and the EC_{50} value should be treated with caution."
Remarks	None

Results

Concentrations	0, 1.0, 10, 100 mg/Kg
Unit	mg/Kg
Results	"The LC_{50} for emergence and EC_{50} for growth rate were both greater that the highest concentration tested, 100 mg/kg, in wheat and mung bean. In radish, the LC_{50} for emergence was greater that the highest concentration tested, 100 mg/kg. Radish seeds exposed to the test material at concentrations of 10 and 100 mg/kg took longer to emerge than control seeds. The EC_{50} for growth was 19 mg/kg (95% confidence limits not calculable due to heterogeneity of results). No other phytotoxic effects were observed for any of the species tested."
Statistical Results	"95% confidence limits not calculable due to heterogeneity of results"
Remarks	None

Conclusions

Remarks: ""The LC_{50} for emergence and EC_{50} for growth rate were both greater that the highest concentration tested, 100 mg/kg, in wheat and mung bean.
In radish, the LC_{50} for emergence was greater that the highest concentration tested, 100 mg/kg. Radish seeds exposed to the test material at concentrations of 10 and 100 mg/kg took longer to emerge than control seeds. The EC_{50} for growth was 19 mg/kg (95% confidence limits not calculable due to heterogeneity of results).
No other phytotoxic effects were observed for any of the species tested."

Data Quality

Remarks: None

References

Inveresk Research, "BuKeto Acid – Terrestrial Plants, Growth Test," July 29, 1998.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guideline for Testing Chemicals No. 209
Test Type	Activated Sludge, Respiration Inhibition Test
GLP (Yes/No)	Yes
Year	1998
Microbial Inoculum	Activated Sludge from Haddington Municipal Sewage Works
Exposure Period	3 hours
Statistical Methods	"The respiration rate over the 2 to 10 minute portion of the measurement period was determined by linear regression. Probit transformation was used for percentage inhibition values. The goodness of fit of the probit model was checked via the Pearson chi-squared test statistic. A significant chi-squared test statistic indicates heterogeneity of discrepancies between the observed and expected percentage inhibition values. Neither test statistic was statistically significant at the 1% level."
Remarks	None

Results

Nominal Concentrations	0.30, 0.95, 3.0, 9.5, and 30 p.p.m.
Unit	p.p.m.
Element Value	"The 3 hour EC_{50} was found to be greater than 30 p.p.m."
Statistical Results	No inhibition at the highest tested concentration.
Remarks	None

Conclusions

Remarks: "The EC_{50}, the concentration at which the respiration rate is 50% of the control respiration rate, was found to be greater than 30 p.p.m. This was selected as the top concentration for the test as it is close to the maximum solubility in water.
The respiration rates for the 2 control vessels were within 15% of each other, and the EC_{50} of the reference material was in the range 5 to 30 p.p.m., meeting the criteria for a valid test."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid – Activated Sludge, Respiration Inhibition Test," April 10, 1998.

Other

None

Health Elements

18. Acute Toxicity

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	Acute Oral Toxicity in rats as specified in the Regulation for the Enforcement of the Federal Hazardous Substances Act (16 CFR 1500)
Test Type	Acute Oral Toxicity - Rats
GLP (Yes/No)	No
Year	1987
Species/Strain	Sprague-Dawley Rat (albino)
Sex	10 male
Number of animals per sex per dose	10 male
Vehicle	The sample material was dosed as a 25% w/v suspension in corn oil.
Route of Administration	Each animal was weighed and dosed by direct administration of the experimental material in the stomach by gavage.

Remarks	● Age: No age given, but rats used weighed 219 – 240 grams ● Doses: 5.0 g/kg ● Doses per time period: One dosage level was administered and the rats were allowed food and water ad libitum, except overnight prior to treatment when food was denied, for the 14 day observation period. ● Volume administered or concentration: 25% w/v in corn oil. ● Post dose observation period: Observed over 14 days, several times during the first day, and once daily thereafter.

Results

Value	LD_{50} > 5.0 g/kg
Number of Deaths at each Dose Level	No deaths occurred during the study.
Remarks	None

Conclusions

Remarks: "The acute oral LD_{50} value was found to be greater than 5.0 g/kg in male Sprague – Dawley rats. The material is not classified as toxic by oral administration."

Data Quality

Remarks: None

References

Hill Top Biolabs, Inc., "Acute Oral Toxicity Screen in Rats," July 6, 1987.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Acute Oral Toxicity test in rats meeting OECD and EEC Guidelines
Test Type	Acute Oral Toxicity - Rats
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Sprague-Dawley Rat
Sex	15 male, 15 female
Number of animals per sex per dose	15 male, 15 female
Vehicle	The sample material was dosed as a suspension in corn oil.
Route of Administration	Each animal was weighed and dosed by direct administration of the experimental material in the stomach by gavage.
Remarks	● Age:6 to 8 weeks old, rats used weighed 153 – 210 grams ● Doses: 1.0, 2.0, 3.0, 4.0, 5.0 g/kg to 2 male and 2 female rats in dose range study. Main study: 5.0 g/kg to 5 male and 5 female rats ● Doses per time period: One dosage level was administered for the 14 day observation period. ● Volume administered or concentration: 10 ml/kg ● Post dose observation period: Observed over 14 days, frequently during the first day, and once daily thereafter.

Results

Value	LD_{50} > 5.0 g/kg
Number of Deaths at each Dose Level	No deaths occurred during the study.
Remarks	None

Conclusions

Remarks: “In the main study, no deaths occurred and no abnormalities were detected at necropsy after oral Administation of BuKeto Acid at a dose level of 5.0 g/kg.
Clinical signs, noted ½ hour after dosing, were confined to one male which showed reduced activity.
The median oral lethal dose (LD_{50}) of BuKeto Acid is greater than 5000 mg/kg.”

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid – Acute Oral Toxicity (LD_{50}) Test in Rats," December 6, 1990.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Acute Dermal Toxicity test in rats meeting OECD and EEC Guidelines
Test Type	Acute Dermal Toxicity - Rats
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Sprague-Dawley Rat
Sex	13 male and 13 female
Number of animals per sex per dose	13 male and 13 female
Vehicle	Undiluted test material
Route of Administration	"The test material was applied evenly onto a gauze dressing which was applied to the shaved back of each rat. Up to at least 10% of the body surface was in contact with the test material. The trunk of the rat was then encircled with a strip of non-irritating tape."
Remarks	● Age: Eight to ten weeks old, rats used weighed 199 - 319 grams ● Doses: 500, 1000, 1500, and 2000 mg/kg for a Dose range study in 2 male and 2 female rats. Main Study: 5000 mg/kg in 5 male and 5 female rats. ● Doses per time period: One dosage per 24 hour contact time period. After the 24 contact period the bandage was removed and the area wiped with cotton wool moistened with water to remove any residual test material. ● Post dose observation period: Observed over 14 days with deaths and overt signs of toxicity recorded.

	Observed frequently after dosing and subsequently once daily for 14 days. Individual body weights were recorded on the day of treatment and on days 7 and 14.

Results

Value	LD_{50} > 2000 mg/kg
Number of Deaths at each Dose Level	No deaths occurred at the 2000 mg/kg dose level
Remarks	None

Conclusions

Remarks: "In the main study, no deaths occurred and no clinical signs were noted after a 24 hour dermal administration, under occlusion, of BuKeto Acid at a dose level of 2000 mg/kg.
The median dermal lethal dose (LD_{50}) of BuKeto Acid in rats is greater than 2000 mg/kg."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid: Acute Dermal Toxicity Test in Rats," December 6, 1990.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(diethylamino)-2-hydroxybenzoyl]

Remarks: EtKeto Acid, (CAS No. 5809-23-4)

Method

Method	Primary Skin Irritation Study in rabbits as specified in the Regulation for the Enforcement of the Federal Hazardous Substances Act (16 CFR 1500)
Test Type	Primary Skin Irritation - Rabbits
GLP (Yes/No)	No

Year	1987
Species/Strain	New Zealand White Rabbits
Sex	3 male, 3 female
Number of animals per sex per dose	3 male, 3 female
Vehicle	"The test material was applied moistened with 0.5 ml of saline."
Route of Administration	"Prior to dosing the application sites were prepared by clipping the hair from the saddle area of the rabbits. The abraded areas were prepared by making minor epidermal incisions with a hypodermic needle. The abrasions were sufficiently deep to penetrate the epidermis, but not to induce bleeding. Each patch was held in place with two strips of one-inch adhesive tape. After application of the patches, the trunk of each rabbit was wrapped with rubber dental dam, which was secured with staples. An outer layer of gauze and tape was placed around the trunk of each animal. The animals were fitted with Elizabethan Collars for approximately 24 hours."
Remarks	● Age: young adult, ● Doses: 0.5 grams ● Doses per time period: One dosage per 24 hour contact time period. ● Post dose observation period: Evaluated the test sites after 24 hours and again after 72 hours. Evaluated for corrosion, erythema, and edema.

Results

Value	Primary Irritation Index: 0.3
Remarks	None

Conclusions

Remarks: "The Primary Irritation Index was found to be 0.3 based on erythema and edema. No evidence of tissue damage was found. The material is not classified as a primary irritant or as a corrosive by dermal application."

Data Quality

Remarks: None

References

Hill Top Biolabs, Inc., "Primary Skin Irritation Acid in Rabbits of EtKeto," July 6, 1987.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Acute Dermal Irritation test in rabbits meeting OECD and EEC Guidelines
Test Type	Acute Dermal Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1990
Species/Strain	New Zealand White Rabbits
Sex	2 male, 1 female
Number of animals per sex per dose	2 male, 1 female
Vehicle	"The test material was applied moistened with 0.5 g of water."
Route of Administration	"The hair was clipped from the dorsal area of the trunk of each rabbit approximately 24 hours before treatment. Care was taken to avoid abrading the skin. The test material (0.5 g, moistened with water), was applied to intact skin on each rabbit under a 2.5 cm x 2.5 cm patch of gauze. The patch was then covered with Micropore tape and the trunk was loosely bound with Elastoplast Elastic Bandage which remained in position for 4 hours. At the end of this period the patches were removed and the skin wiped with water dampened tissues to remove surplus test material without altering the existing response or the integrity of the epidermis."
Remarks	● Age: young adult ● Doses: 0.5 grams ● Doses per time period: One dosage per 4 hour contact time period. ● Post dose observation period: Skin reactions were assessed 1, 24, 48, and 72 hours after patch removal.

Results

Value	"BuKeto Acid is non-irritant to rabbit skin."
Remarks	None

Conclusions

Remarks: "No skin reactions were noted following a 4 hour semi-occlusive application of BuKeto Acid to rabbit skin. BuKeto Acid is non-irritant to rabbit skin."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid: Acute Dermal Irritation Test in Rabbits," December 6, 1990.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Acute Eye Irritation Test in rabbits meeting OECD and EEC Guidelines
Test Type	Acute Eye Irritation - Rabbits
GLP (Yes/No)	Yes
Year	1990
Species/Strain	New Zealand White Rabbits
Sex	1 male, 2 female
Number of animals per sex per dose	3 rabbits – 1 male, 2 female
Vehicle	Undiluted test material
Route of Administration	"Approximately 24 hours before test commencement, both eyes of rabbits were examined and only animals

	with no ocular defects were used in the test. The quantity of material instilled into the treated eye was 100 mg. Instillation of the test material was by the following technique: The rabbit was held firmly but gently and the test material placed into the right eye by gently pulling the lower eyelid away from the eyeball to form a sac into which the test material was dropped. The lids were then gently held together for one or two seconds. The other eye remained untreated to serve as a control."
Remarks	● Age: young adult ● Doses: 100 mg ● Doses per time period: One dosage per 72 hour observation period. ● Post dose observation period: Assessment of damage/irritation was made 1, 24, 48, and 72 hours following treatment.

Results

Value	"BuKeto Acid is practically non-irritant to rabbit eyes."
Remarks	None

Conclusions

Remarks: "Slight conjuctival redness was noted in all treated eyes 1 hour post instillation, persisting in one eye until 24 hours when a slight discharge was also noted. All treated eyes were normal by 48 hours post instillation. BuKeto Acid is practically non-irritant to rabbit eyes."

Data Quality

Remarks: None

References

Inveresk Research International, "Buketo Acid: Acute Eye Irritation Test in Rabbits," December 6, 1990.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Maximization Technique (Magnusson and Kligman), satisfies OECD Guidelines
Test Type	Magnusson-Kligman Maximization Test in Guinea Pigs
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Dunkin-Hartley albino guinea pigs
Sex	48 female
Number of animals per sex per dose	48 female guinea pigs
Vehicle	Paraffin oil
Route of Administration	Injection Induction: 10%, 5%, 2%, and 1% w/v BuKeto Acid in paraffin oil at 0.1 ml Topical Induction: 25%, 10%, 5%, and 2% w/v BuKeto Acid in paraffin oil at 0.1 ml Primary Challenge: 25% and 10% w/v BuKeto Acid in paraffin oil at 0.1 ml
Remarks	● Age: young adult, Guinea pigs used weighed 411 – 503 grams ● Doses: Injection: 0.1 ml, Topical: 0.1 ml, Challenge: 0.1 ml ● Doses per time period: One dosage per 24 to 48 hour observation period. ● Post dose observation period: Assessment of damage/irritation was made 24, 48 hours following treatment.

Results

Value	"BuKeto Acid is classified as a weak sensitiser according to the Magnusson-Kligman classification"
Remarks	None

Conclusions

Remarks: "There is no evidence from the test results that BuKeto Acid is a sensitiser in guinea pigs. BuKeto Acid is classified as a weak sensitiser according to the Magnusson-Kligman classification."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid: Magnusson-Kligman Maximization Test in Guinea Pigs," December 6, 1990.

Other

None

Genetic Toxicity Elements

19. Genetic Toxicity In Vivo

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	*In Vivo* Rat Liver Unscheduled DNA Synthesis Assay (Butterworth BE, Ashby J, Bermudez E, Casciano D, Mirsalis J, Probst G, Williams GM, 1987), Mutation Research,189, 123-133
Test Type	*In Vivo* Rat Liver Unscheduled DNA Synthesis Assay
GLP (Yes/No)	Yes
Year	1998
Species/Strain	Male Alderley Park (Alpk: Ap_fSD) rats
Sex	male
Route of Administration	Single oral dose
Doses/concentration levels	3200 and 5000 mg/kg
Exposure period	2 hours and 16 hours
Statistical methods	"The computer system calculated the mean nuclear grain count [N], the mean cytoplasmic grain count [C], the mean net nuclear grain count [N-C] and the percentage of cells in repair (i.e. cells with N-C values of at least 5) for each slide, animal and treatment group."
Remarks	● Age: Six to seven weeks old ● No. animals per dose: 5 rats at 3200 mg/kg and 16 hours, 5 rats at 5000 mg/kg and 16 hours, 5 rats at 3200 mg/kg and 2 hours, 5 rats at 5000 mg/kg and 2 hours ● Vehicle: Corn oil

	● Duration of test: 4 days ● Frequency of treatment: Single oral dose ● Control groups and treatment: Corn oil vehicle control (20 ml/kg) at 16 hours and 2 hours, 1,2-dimethylhydrazine dihydrochloride (positive control) (30 ml/kg) at 16 hours and 2 hours ● Clinical observations: Signs of coloration or abnormalities to organ/tissues ● Organs examined at necropsy: Liver tissue ● Criteria for evaluating results: Nuclear grain count, mean cytoplasmic grain count, mean net nuclear grain count, and the percentage of cells in repair for each slide, animal and treatment group ● Criteria for selection of M.T.D.: "The maximum tolerated dose (MTD) was selected as 5000 mg/kg which is the limit dose for the assay."

Results

UDS Assay and Statistical results	"Signs of diarrhea was observed for some rats dosed at 3200 mg/kg and 5000 mg/kg. One rat dosed at 3200 mg/kg showed signs of urinary incontinence. Mean viablities of the hepatocyte cultures ranged from 73.2% to 77.7%. Hepatocytes prepared from all animals were examined microscopically. No apparent signs of excessive cytotoxicity (e.g. few cells present, a high proportion of cells of abnormal morphology or large number of pyknotic cells) were observed on slides from animals dosed with BuKeto Acid. Slides from animals treated with BuKeto Acid at both dose levels were therefore assessed for UDS. BuKeto Acid caused no significant increases, compared to the vehicle control, in mean net nuclear grain count, or in percentage of cells in repair, at either dose level or time point investigated. Hepatocytes from BuKeto Acid treated animals had mean net nuclear grain values of less than 0. These data therefore provided no evidence for induction of UDS by BuKeto Acid."
Remarks	"The sensitivity of the test system was clearly demonstrated by the marked increases in DNA repair (as measured by UDS) induced in the positive control substance, 1,2-dimethylhydrazine dihydrochloride."

Conclusions

Remarks: "Under the conditions of the test, BuKeto Acid did not induce DNA repair (as measured by unscheduled DNA synthesis) in rat liver *in vivo*."

Data Quality

Remarks: None

References

Central Toxicology Laboratory, "BuKeto Acid: In Vivo Rat Liver Unscheduled DNA Synthesis Assay," August 7, 1998.

Other

None

20. Genetic Toxicity In Vitro

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
Test Type	Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells (Cytogenetic assay)
System of Testing	Chinese Hamster Ovaries
GLP (Yes/No)	Yes
Year	1991
Species/Strain	Chinese Hamster Ovary (CHO-10 B_4), State University of Leiden, Netherlands
Metabolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Fischer 344 rat) Quantity: 50 µl at 0.5 ml Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	Presence of S-9 Mix: 5, 10, 20 and 30 µg/ml Absence of S-9 Mix: 25, 50, 75, and 100 µg/ml
Statistical Methods	"Statistical evaluation of in-house historical data from vehicle and untreated control cultures has enabled acceptable aberration frequency ranges for a negative

	response to be defined. A negative test substance response was recorded if the measured aberration parameter fell within 95% confidence limits of mean historical values of vehicle control cultures. A positive response was recorded whenever aberration incidences in treated cultures repeatedly equaled or rose above the upper 99% confidence limits of mean historical values of vehicle control cultures. Importance was also placed on the demonstration of dose related and reproducible increases in the assessed aberration parameters. Sporadic increases in structural aberrations in compound treated cultures whether over the 95% or 99% confidence levels were discussed individually. Where control values fell between 95 and 99% limits, the frequency was deemed elevated. The responses to the test and positive control substances were then judged as positive if a doubling over these elevated control frequencies were achieved. A test was rejected if vehicle or medium-only control values fell outside the upper 99% confidence limits for 2 of the 3 measured aberration parameters. Similarly, a test was rejected if positive control values (for at least one positive control) were not in excess of the upper 99% confidence limits for 2 of the 3 measured parameters shown."
Remarks	None

Results

Cytotoxic concentration	With metabolic activation: "In the presence of S9 mix BuKeto Acid was a potent inducer of chromosomal aberrations when tested at toxic concentrations of 20 and 30 µg/ml." Without metabolic activation: no test concentration caused aberrations
Statistical Results	"In the presence of S9 mix BuKeto Acid was a potent inducer of chromosomal aberrations when tested at toxic concentrations of 20 and 30 µg/ml. This response was dose related. There was no evidence that BuKeto Acid induced chromosomal aberrations in the absence of the S9 mix."
Remarks	"Concurrent vehicle and positive control cultures demonstrated the sensitivity of the test system." The solvent control used was cyclophosphamide (CPH). The test article solvent vehicle was dimethyl sulfoxide (DMSO).

Conclusions

Remarks: “It was concluded that BuKeto Acid was clastogenic *in vitro* when tested for such effects, to toxic concentrations, in the presence of S9 mix with an established Chinese hamster ovary (CHO) cell line.”

Data Quality

Remarks: None

References

Inveresk Research International, “BuKeto Acid: Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*” July 12, 1991.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	*Salmonella*/Mammalian-Microsome Mutagenicity Assay (McCann *et al*., 1975; McCann and Ames, 1975)
Test Type	Testing for Mutagenic Activity with *Salmonella typhimurium*
System of Testing	*Salmonella typhimurium*
GLP (Yes/No)	Yes
Year	1991
Species/Strain	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, and TA 1538
Metabolic Activation	Species and cell type: Aroclor 1254 – induced rat liver S-9 (male Fischer 344 rat) Quantity: 0.5 ml to 2 ml of molten agar Induced or not induced: Studied induced and non-induced cells
Concentrations Tested	Toxicity test: 33, 100, 333, 1000, 3333, 10000 µg per plate Mutation test: 3, 10, 33, 100, 333, 1000 µg per plate

Statistical Methods	For all replicate platings, the mean revertants per plate and the standard deviation will be calculated.
Remarks	None

Results

Genotoxic effects concentration	"No mutagenic activity was observed in any of the 5 bacterial strains used, either in the presence or absence of S9 mix. Toxicity to the bacteria was observed 1000 µg per plate in both activation systems.
Statistical Results	No appreciable toxicity was observed. No positive responses were observed.
Remarks	The test article solvent vehicle was dimethyl sulfoxide (DMSO). The results obtained in the positive control groups were within the normal ranges expected for each bacterial strain and activation system.

Conclusions

Remarks: "It was concluded that BuKeto Acid was not mutagenic *to Salmonella typhimurium* when tested in dimethylsulphoxide at concentrations extending into the toxic range."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid: Testing for Mutagenic Activity with *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, and TA 100," August 31, 1990.

Other

None

21. Repeated Dose Toxicity

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Four Week Oral Toxicity Study in Rats conforms with Annex V, published in the Official Journal of the European Communities (no. L251, 19, September 1984)
Test Type	28 Day Oral Toxicity Study in Rats
GLP (Yes/No)	Yes
Year	1990
Species/Strain	Sprague-Dawley Rats
Route of Administration	The test substance was administered once daily orally via a steel cannula at a constant dose volume of 5 ml dosing suspension per kg body weight.
Duration of test	28 days
Doses/concentration levels	Dose volume: 5 ml/kg/day Dose Levels: 0, 50, 250, and 1000 mg/kg/day
Sex	23 male and 23 female
Exposure period	Animals were treated once daily, seven days per week for four weeks.
Frequency of treatment	The test substance was administered once daily orally via a steel cannula at a constant dose volume of 5 ml dosing suspension per kg body weight.
Control group and treatment	Control animals similarly received 5 ml per kg body weight of corn oil (vehicle).
Post exposure observation period	"Viability was checked once each morning and once as late as practicable on each day. All animals were examined for reaction to treatment during each day. The onset, intensity and duration of these signs were recorded. All animals received a detailed clinical examination once each week. The weight of each animal was recorded weekly. The quantity of food consumed by each cage of animals was recorded once each week. Water consumption was monitored by visual inspection on a weekly basis thought the study. All animals were killed and necropsied."
Statistical methods	"Haematology, clinical chemistry, organ weight and body weight data were statistically analyzed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogenous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous log or square root transformations were used in an attempt to stabilize the variances. If the variances remained heterogeneous then a non-parametric test such as Kruskal-Wallis ANOVA was

	used. Organ weights were also analyzed conditional on body weight (i.e. analysis of covariance). Histology data were analyzed by Fisher's Exact Probability test."
Remarks	● Age: 4 weeks old, rats used weighed: male – *ca* 85 grams, female – *ca* 60 grams ● No. of animals per sex per dose: 23 males, 23 females ● Vehicle: corn oil ● Clinical observations performed and frequency: All animals were observed daily for reactions to treatment. ● Organs examined at necropsy: adrenals, heart, kidneys, liver, spleen, testes, ovaries, any other macroscopically abnormal tissue

Results

NOAEL (NOEL)	"There were no notable effects seen at 50 mg/kg/day or 250 mg/kg/day in males or at any dose level in females."
LOAEL (LOEL)	"BuKeto Acid produced a moderate reduction in body weight gain with a concomitant slight reduction in food consumption at 1000 mg/kg/day in males only."
Actual dose received by dose level by sex	0, 50, 250, and 1000 mg/kg/day
Toxic response/effects by dose level	"There was a moderate reduction in body weight gain in the male high dose group. There was a slight reduction in total food consumed in the male high dose group."
Statistical results	"There was a moderate reduction in body weight gain in the male high dose group. There was a slight reduction in total food consumed in the male high dose group."
Remarks	● Body weight: There was a moderate reduction in body weight gain in the male high dose group ● Food/water consumption: There was a slight reduction in total food consumed in the male high dose group ● Description, severity, time of onset and duration of clinical signs: No notable differences in either sex ● Ophthalmologic findings incidence and severity: No notable differences in either sex ● Hematological findings incidence and severity: No notable differences in either sex ● Clinical biochemistry findings incidence and severity:

	No notable differences in either sex ● Mortality and time to death: No notable differences in either sex ● Gross pathology incidence and severity No notable differences in either sex ● Organ weight changes: No notable differences in either sex ● Histopathology incidence and severity: No notable differences in either sex

Conclusions

Remarks: "Dosing Sprague-Dawley rats for 4 weeks with BuKeto Acid produced a moderate reduction in body weight gain with a concomitant slight reduction in food consumption at 1000 mg/kg/day in males only.
There were no notable effects seen in 50 mg/kg/day or 250 mg/kg/day in males or at any dose level in females."

Data Quality

Remarks: None

References

Inveresk Research International, "BuKeto Acid: 4 Week Oral Toxicity Study in Rats," November 30, 1990.

Other

None

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Thirteen Week Oral Toxicity Study in Rats conforms with OECD Guidelines
Test Type	13 Week Oral Toxicity Study in Rats
GLP (Yes/No)	Yes
Year	1998
Species/Strain	Sprague-Dawley Rats (CD BR)

Route of Administration	The test substance was administered once daily by the oral gavage route, 7 days per week for 13 consecutive weeks.
Duration of test	13 weeks
Doses/concentration levels	Dose volume: 5 ml/kg/day Dose Levels: 0, 50, 250, and 1000 mg/kg/day
Sex	43 male and 43 female
Exposure period	Animals were treated once daily, seven days per week for thirteen weeks.
Frequency of treatment	The test substance was administered once daily orally by gavage at a constant dose volume of 5 ml dosing suspension per kg body weight.
Control group and treatment	Control animals similarly received 5 ml per kg body weight of corn oil (vehicle).
Post exposure observation period	"Viability was checked once each morning and once as late as practicable on each day. All animals were examined for reaction to treatment during each day. The onset, intensity and duration of these signs were recorded. All animals received a detailed clinical examination once each week. The weight of each animal was recorded weekly. The quantity of food consumed by each cage of animals was recorded once each week. Water consumption was monitored by visual inspection on a weekly basis thoughout the study. However, after an observation on week 7 the water consumption was recorded from week 8 to the end of the study. All animals were killed and necropsied."
Statistical methods	"Body weight, food consumption, haematology, coagulation, urinalysis, clinical chemistry, and organ weight data were statistically analyzed for homogeneity of variance using the 'F-max' test. If the group variances appeared homogenous a parametric ANOVA was used and pairwise comparisons made via Student's t-test using Fisher's F-protected LSD. If the variances were heterogeneous log or square root transformations were used in an attempt to stabilize the variances. If the variances remained heterogeneous then a non-parametric test such as Kruskal-Wallis ANOVA was used. Organ weights were also analyzed conditional on body weight (i.e. analysis of covariance). Histology data were analyzed by Fisher's Exact Probability test."
Remarks	● Age: 4 weeks old, rats used weighed: male – *ca* 83-

	87 grams, female – *ca* 58-62 grams ● No. of animals per sex per dose: 43 males, 43 females ● Vehicle: corn oil ● Clinical observations performed and frequency: All animals were observed daily for reactions to treatment. ● Organs examined at necropsy: adrenals, heart, kidneys, liver, spleen, testes, ovaries, brain, eye, gastro-intestinal tract, lung, pancreas, pituitary, prostrate, skin, spinal cord, sternum, thigh muscle, thymus, thyroid, tongue, trachea, bladder, uterus, vagina, any other macroscopically abnormal tissue

Results

NOAEL (NOEL)	"There were no signs of toxicity at 50 mg/kg/day, which was therefore classed as the No Toxic Effect Level."
LOAEL (LOEL)	"At 250 mg/kg/day males and females showed signs of salivation and body weight gain reduction as well as increased water consumption in females only."
Actual dose received by dose level by sex	0, 50, 250, and 1000 mg/kg/day
Toxic response/effects by dose level	"No treatment related differences were seen in haematology, clinical chemistry and urinalysis parameters or in post mortem investigations. Males and females receiving 1000 mg/kg/day showed signs of gastro-intestinal disturbance and salivation, body weight gain was slightly reduced and water consumption was increased in both sexes. At 250 mg/kg/day males and females showed signs of salivation and body weight gain reduction as well as increased water consumption in females only."
Statistical results	"No treatment related differences were seen in haematology, clinical chemistry and urinalysis parameters or in post mortem investigations. Males and females receiving 1000 mg/kg/day showed signs of gastro-intestinal disturbance and salivation, body weight gain was slightly reduced and water consumption was increased in both sexes. At 250 mg/kg/day males and females showed signs of salivation and body weight gain reduction as well as increased water consumption in females only."
Remarks	● Body weight: Body weights and gains were slightly reduced at 250 and 1000 mg/kg/day as compared to controls in males

	● Food/water consumption: No notable differences in either sex. There was a moderate increase in water consumption at 1000 mg/kg/day in males and at 250 and 1000 mg/kg/day in females ● Description, severity, time of onset and duration of clinical signs: No notable differences in either sex ● Ophthalmologic findings incidence and severity: No notable differences in either sex ● Hematological findings incidence and severity: No notable differences in either sex ● Clinical biochemistry findings incidence and severity: No notable differences in either sex ● Mortality and time to death: No notable differences in either sex ● Gross pathology incidence and severity No notable differences in either sex ● Organ weight changes: No notable differences in males. Slight increase in thyroid and liver weight in males. Not substantiated by histological lesion. ● Histopathology incidence and severity: No notable differences in either sex

Conclusions

Remarks: “It was concluded from observations, laboratory investigations and terminal studies that dosing Sprague-Dawley rats orally by gavage for 13 weeks at levels of 250 and 1000 mg/kg/day produced only signs of mild toxicity attributable to BuKeto Acid.”

Data Quality

Remarks: None

References

Inveresk Research International, “BuKeto Acid: 13 Week Oral Toxicity Study in Rats with Administration by Gavage,” October 2, 1998.

Other

None

22. Toxicity to Reproduction

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	OECD Guidelines for Testing Chemicals No. 415
Test Type	One generation reproductive test
GLP (Yes/No)	Yes
Year	1999
Species/Strain	Sprague-Dawley rats
Route of Administration	Doses were administered orally by gavage at a dose volume of 5 ml dosing solution per kg body weight, using a steel dosing cannula.
Doses/concentration levels	Dose volume: 5 ml/kg bodyweight (Suspension in corn oil) Dose Levels: 0 (Control), 50, 250, and 1000 mg/kg/day
Sex	104 male and 104 female
Control group and treatment	24 male and 24 female Dose: 5 ml/kg bodyweight (corn oil)
Frequency of treatment	The once daily doses were administered ten weeks prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring.
Duration of test	21 weeks (21 day post partum)
Premating exposure period for males	10 weeks
Premating exposure period for females	10 weeks
Statistical methods	"Where required to assist the interpretation, tests were applied to determine the statistical significance of observed differences between controls and treated groups. Organ weight data were analyzed by analysis of variance and by analysis of covariance using the terminal body weight as the single covariate. Pairwise comparisons between each treatment level and control were performed using Fisher's F-Protected T-test. For other parameters, interpretation was based on examination of the individual group values."
Remarks	● Age: 4 weeks old, males used weighed 61 - 96 grams, females weighed: 53 - 82 grams ● No. of animals per sex per dose: 24 males, 24 females ● Vehicle: corn oil

	● Dosing schedule and pre and post dosing observation periods: The once daily doses were administered ten weeks prior to pairing, through pairing, pregnancy, and lactation up to sacrifice after weaning of their offspring. Observed through 21 day post partum. (21 weeks) ● Mating procedures (M/F ratios per cage, length of cohabitation, proof of pregnancy): One male and one female, 7 night mating period, daily vaginal smears to determine proof of pregnancy ● Standardization of litters: "The females were allowed to litter normally. The day of birth of the litter (day on which parturition commenced) was designated Day 0 of lactation. The duration of gestation in days was evaluated. The number of live pups born and the number found dead in each litter was recorded as soon as possible after completion of parturition, but it was ensured that disturbance of the mother and the letter was minimized at this sensitive time. The live pups were sexed, counted, examined for the presence of milk in the stomach and for any externally visible abnormality on Days 7, 14, and 21 of lactation. Where practicable, any pups found dead of killed during lactation were sexed and examined above." ● Clinical observations performed and frequency: "All the animals were examined for reaction to treatment on each day. The nature, onset, duration and intensity of any signs were recorded. Additionally, following observations made during the first two weeks of treatment, a 4 hour after dosing check was made on all animals during the remainder of the treatment period. A detailed examination was performed weekly which included appearance, movement and behavior patterns, skin and hair condition, eyes and mucous membranes, respiration, and excreta. In addition, all the animals were checked for viability at the beginning of each day and again as late as possible on each day." Body weights: once per week, Food consumption: recorded weekly, Necropsy organs taken and fixed: ovaries, Uterus, cervix, vagina, testes, epididymides, seminal vesicles and coagulating gland, prostrate gland, pituitary gland.

Results

NOAEL (NOEL)	"There were no reproductive effects detected at 50 mg/kg/day."

LOAEL (LOEL)	"Under the conditions of this study, parental toxicity and reproductive effects were observed at 250 and 1000 mg/kg/day."
Actual dose received by dose level by sex	"The analyzed concentrations of dosing suspensions were within ± 10% of the nominal concentration, indicating acceptable accuracy of formulation."
Parental data, descriptions	"Under the conditions of this study, parental toxicity and reproductive effects were observed at 250 and 1000 mg/kg/day."
Offspring toxicity	"At 250 and 1000 mg/kg/day, mean litter weights were lower than control, although mean pup weights were only slightly lower at 1000 mg/kg/day and increased at 250 mg/kg/day (reflecting the smaller litter sizes). It was considered that there had probably been a marginal effect of treatment on litter/pup weights at these levels."
Toxic response/effects by dose level	"Mean body weight gain of males was reduced at 1000 and 250 mg/kg/day. There was a slightly reduced initial weight gain at 1000 mg/kg/day. Clinical signs of reaction at these levels included unkempt/greasy coats and soft feaces. At 1000 mg/kg/day, reduced weight gain was observed during gestation. All pups in 8/21 litters at this level died or were killed, and 3 dams were killed at around the same time; a further dam was killed during parturition. In the surviving litters, pup survival was similar to control. At 250 mg/kg/day, weight gain during gestation was slightly reduced. The incidence of litters in which all pups died was similar to control, but pup mortality in the surviving litters was increased. At 50 mg/kg/day, pup mortality was similar to control. At 250 and 1000 mg/kg/day, mean litter weights were lower than control, although mean pup weights were only slightly lower at 1000 mg/kg/day and increased at 250 mg/kg/day (reflecting the smaller litter sizes). It was considered that there had probably been a marginal effect of treatment on litter/pup weights at these levels."
Statistical results	"Mean body weight gain of males was reduced at 1000 and 250 mg/kg/day. There was a slightly reduced initial weight gain at 1000 mg/kg/day. Clinical signs of reaction at these levels included unkempt/greasy coats and soft feaces. At 1000 mg/kg/day, reduced weight gain was observed during gestation. All pups in 8/21 litters at this level died or were killed, and 3 dams were killed at around the same time; a further dam was killed during parturition.

	In the surviving litters, pup survival was similar to control. At 250 mg/kg/day, weight gain during gestation was slightly reduced. The incidence of litters in which all pups died was similar to control, but pup mortality in the surviving litters was increased. At 50 mg/kg/day, pup mortality was similar to control. At 250 and 1000 mg/kg/day, mean litter weights were lower than control, although mean pup weights were only slightly lower at 1000 mg/kg/day and increased at 250 mg/kg/day (reflecting the smaller litter sizes). It was considered that there had probably been a marginal effect of treatment on litter/pup weights at these levels."
Remarks	● Body weight: "Mean body weight gain of males was reduced at 1000 and 250 mg/kg/day. There was a slightly reduced initial weight gain at 1000 mg/kg/day. Clinical signs of reaction at these levels included unkempt/greasy coats and soft feaces. At 1000 mg/kg/day, reduced weight gain was observed during gestation. All pups in 8/21 litters at this level died or were killed, and 3 dams were killed at around the same time; a further dam was killed during parturition. In the surviving litters, pup survival was similar to control. At 250 mg/kg/day, weight gain during gestation was slightly reduced. The incidence of litters in which all pups died was similar to control, but pup mortality in the surviving litters was increased. At 250 and 1000 mg/kg/day, mean litter weights were lower than control, although mean pup weights were only slightly lower at 1000 mg/kg/day and increased at 250 mg/kg/day (reflecting the smaller litter sizes). It was considered that there had probably been a marginal effect of treatment on litter/pup weights at these levels." ● Food/water consumption: Similar to those of control animals ● Fertility index: : Similar to those of control animals ● Duration of gestation: Similar to those of control animals ● Gestation index: Similar to those of control animals ● Mortality: The incidence of deaths at 100 mg/kg/day was much greater than would be expected in Control animals and therefore the deaths at this level were attributed to BuKeto Acid. ● Gross pathology incidence and severity: Similar to those of control animals

	● Ovarian weight changes: Similar to those of control animals ● Offspring toxicity: At 1000 mg/kg/day pup mortality was indicated by the loss of complete litters, but 250 mg/kg/day the susceptible litters only lost some of their pups. ● Litter size and weights: At 250 and 1000 mg/kg/day, mean litter weights were lower than control, although mean pup weights were only slightly lower at 1000 mg/kg/day and increased at 250 mg/kg/day (reflecting the smaller litter sizes). It was considered that there had probably been a marginal effect of treatment on litter/pup weights at these levels. ● Organ weights: Similar to those of control animals

Conclusions

Remarks: "Under the conditions of this study, parental toxicity and reproductive effects were observed at 250 and 1000 mg/kg/day. There were no reproductive effects detected at 50 mg/kg/day."

Data Quality

Remarks: None

References

Inveresk Research, "BuKeto Acid: One Generation Reproduction Study in Rats," March 25, 1999.

Other

None

Toxicokinetic Assessment

23. Toxicokinetic Assessment

Test Substance

Identity: Benzoic acid, 2-[4-(dibutylamino)-2-hydroxybenzoyl

Remarks: BuKeto Acid, (CAS No. 54574-82-2)

Method

Method	Assessment of the adsorption, distribution, metabolism, and excretion of BuKeto Acid
GLP (Yes/No)	No
Year	2001
Studies reviewed	Physico-chemical properties, Acute Oral Toxicity, Acute Dermal Toxicity, Skin Irritation, Eye Irritation, Subacute Toxicity (28 day test), One Generation Reproduction Study, 13 Week Oral Toxicity in Rats, Rat Hepatocyte UDS Assay, Mutagenicity
Remarks	None

Results

Assessment	"Lack of clinical signs in the toxicity tests may indicate poor absorption of lack of inherent toxicity. The substance is a weak acid. At the pH in the stomach (*ca* 1.4) it will be non-ionized and highly lipid soluble, therefore absorption may occur. In other regions of the gastro-intestinal tract, where the pH is higher, absorption is likely to be substantially reduced. At skin pH (*ca* 5.5) the partition coefficient of the substance will be quite high (Log P_{ow} *ca* 5) and absorption into the systemic circulation is unlikely. Although it may penetrate the outer layers of the stratum corneum. Once in the blood stream at plasma pH, the substance is likely to be ionized, although the log P_{ow} at pH 7 (2.67) indicates that this still has appreciable soluble lipid solubility. This indicates that, once absorbed into the bloodstream, the substance may be capable of partitioning into fatty tissues and possibly remaining there. However no evidence of tissue abnormalities was observed in the studies."
Remarks	None

Conclusions

Remarks: "At pH 7, the substance has reasonable water solubility but still greater affinity for lipid media rather than aqueous (log P_{ow} is 2.67). The substance may be excreted unchanged via the kidney, however, metabolism to form more water soluble/polar compounds which could result in more rapid excretion is likely. Demethylation or dealkylation of the side chains may occur, while N-dealkylation is also possible. The linked aromatic rings may undergo phase II conjugation reactions, with the hydroxyl mostly conjugated to glucuronide and the carboxyl to glucuronide or glycine."

Data Quality

Remarks: None

References

Inveresk Research, “Toxicokinetic Assessment of BuKeto Acid,” January 10, 2001

Other

None